References:

① Basic Fuchsin as a Stain for Osmium-Fixed, Epon-Embedded Tissue.

Winkelstein, Jerry ; Menefee, Max G. and Bell, Allen

Stain Technology 38 - 202-204 (1963)

② Richardson, K.C. ; L. Jarett and E.H. Fi

Embedding in Epoxy resins for ultra thin sectioning in electron microscopy.

Stain Technology 35, 313-323 (1960)

③ Luft, J. H. (1961)

Improvement in epoxy resin embedding methods

J. Biophysical, Biochemical Cytology 9, 409-

④

Reference to Stains:

(1) Basic Fuchsin C.I. 42500
Allied Chemical Company

(2) Methylene Blue (methylthionine Chloride)
Merk & Co.

Observations:

(1) Staining times longer than 1 min. in Basic Fuchsin will cause the stain to be bound to tissue components more tightly thus requiring more de staining time in the dist. water and also longer staining times in the methylene blue

(2) Varying the concentration of sodium hydroxide with methylene blue stain.

- .5N NaOH — sections removed from slide
- .2N NaOH — irregular staining - dark patches (probably the NaOH is causing ares of sections to come loose from slide and stain more intensely)
- .1 N NaOH - Good staining
- .05N NaOH - good but takes longer time

(3) pH of Solutions

methylene blue = 2.5 - 3 — Is Meth Blue a Basic Dye?
.1N sodium hydroxide = 12.5

combination of above 1:1 = 12.5 pH

(4) Mixtures of sod. hydroxide and mehylene blue

1 part M.B. + 1 part NaOH = good stain
1 " " + 2 " " = " "
2 " " 1 " " = longer stain time
5 " " 1 " " = very little staining

⑤ If the methylene blue and sod. hydroxide are mixed they will begin to form a precipitate, so the two solutions are not mixed prior to staining. The mixed solution is good for about 1/2 hour.

Can use M. Blue 1st → then NaOH – stain OK. However if need stain more → add " " → then loses blue stain?

⑥ The sod. hydroxide should be placed on the slide first, because the methylene blue as an acid will remove any previously blue stained parts of the cell when attempting to restain with methylene blue to darken the section

5 drops conc. / 20ml H_2O

⑦ An acid solution of HCL at about pH 3 may be used on sections after basic fuchsin staining to remove more stain from certain areas and give more contrast. This should not replace the dist. water treatment which should come first, because the HCL will not remove any of the background staining. Collagen, elastic, stratum corneum

⑧ For some tissues and thin sections the destain time with dist. H_2O may have to be shortened so too much stain is not removed.

⑨ If staining times in methylene blue ~~are~~ must be shorter than two minutes to achieve results the normality of the sod. hydroxide should be adjusted down so that longer staining times may be used.

Solutions:

① Basic Fuchsin 4-5% basic fuchsin in dist. water.

This solution is placed on the staining hot plate at 70°C. It seems best to make this solution up fresh each day or if kept longer to filter occasionally.

② Methylene blue 1-2% methylene blue in dist H_2O.

③ Sodium Hydroxide 0.1 N Sodium Hydroxide in dist H_2O.

Procedure:

① Place slide on hot plate at 70°C.

② Add a few drops of basic fuchsin stain to sections. Stain 1 min.

③ Rinse slide & water until all excess stain is removed from slide

④ Place slide back on hot plate and add a few drops of dist. H_2O to sections
De stain for 1 min.

⑤ Rinse and dry.

⑥ Being sure slide is at room temp. add 2parts of sod. hydroxide and then 1part methylene to sections. Agitate slide for 15 sec. to mix solutions. Stain for 2-3 min.

⑦ Rinse quickly in cold water and dry (use forced air to blow dry or filter paper)

⑧ Mount - since most mounting mediums cause rapid fading, we use one drop of Zeiss immersion oil, add # 1½ cover slip and when oil reaches edges of cover slip. Ring the coverslip with a small amount of Sear's epoxy cement (Hardener + resin). The slide can the be placed in a 60° oven, for ½-1 hour